tetrahydrobiopterin (BH₄) plays an important role in functional and metabolic cellular homeostasis, with additional effects on proliferation, and immune responsiveness. Mutations in either de novo biosynthetic or regeneration (salvage) pathways result in BH₄ deficiency associated with diminished levels of serotonin and dopamine with progressive neurologic symptoms. The phenotypic presentation of these synthetic mutations can be predicted in large part by the role of BH₄ as an obligatory cofactor in phenylalanine, tryptophan, and tyrosine hydroxylases (the rate-limiting enzymes for catecholamine and serotonin synthesis). The function of BH₄ in these aromatic amino acid hydroxylases involves redox-active donation of electrons and reductive enzyme activation and is associated with a tightly coupled system for regeneration of BH₄ from the oxidized dihydrobiopterin. More recently, partially oxidized analogues of BH₄ have been shown to also enhance rates of superoxide formation from purified eNOS in the presence of saturating L-arginine concentrations, suggesting that the ratio of reduced and oxidized biopoterin may be physiologically important in determining rates of NO production versus uncoupled superoxide formation from eNOS.

The increase in oxidant formation that appears to accompany most, if not all, disease processes associated with endothelial-dependent vascular dysfunction may potentially be a result of inadequate BH₄ concentrations within the vasculature, with ensuing uncoupling of eNOS, increased superoxide formation and diminished NO formation. Thus, there is intense interest in the potential for BH₄ to modulate eNOS activity. Numerous groups have recently reported beneficial effects of acute (≤60 minutes) administration of BH₄ or analogs such as sepiapterin on endothelial function in both clinical and experimental models of hypercholesterolemia, atherosclerosis, hypertension, and cigarette smoking. Additionally, the beneficial effects of vitamin C administration on endothelial function are in part due to stabilization of intracellular BH₄ with resultant enhanced NO bioactivity. A similar mechanism of BH₄ stabilization may underlie the influence of folicate supplementation on eNOS activity.

Alterations in BH₄-dependent eNOS activity have also been implicated in diabetic vascular dysfunction where sepiapterin has been reported to diminish eNOS-derived superoxide in human vascular segments. Acute administration of a BH₄ analog augmented endothelial-dependent relaxation in a streptozotocin model of diabetes. Spontaneously diabetic BB rats have diminished GTP-cyclohydrolase I activity and decreased BH₄ levels, while sepiapterin treatment for 48 hours normalized BH₄ levels and increased NO production from endothelial cells isolated from diabetic BB rats. In an insulin-resistant rat model, chronic oral administration of BH₄ also increased vascular BH₄ content and improved endothelial-dependent vascular function. Given these positive outcomes after administration of BH₄, either longer term in vitro exposures or chronic administration studies are warranted in models of atherosclerosis.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Vasquez-Vivar et al report their interesting findings on the influence of 6 hours sepiapterin incubation on vascular BH₄ levels and endothelial function in vessels isolated from rabbits fed a high-cholesterol diet. They demonstrate a marked reduction in vascular BH₄ content in hyperlipidemic vessels compared with controls, which could be restored by incubation with sepiapterin. These findings are in agreement with the relatively few other studies reporting on BH₄ content in diseases associated with vascular dysfunction. While vascular BH₄ levels have been reported to be...
normal in spontaneously hypertensive rats (SHR) before the development of hypertension, BH$_4$ levels are decreased in SHR with established hypertension.$^{30,31}$ Several models of diabetes have also reported decreased vascular BH$_4$ content.$^{27,28}$

In contrast to the improvement of endothelial function seen in acute studies, however, long-term exposure to sepiapterin resulted in a further derangement in response to either acetylcholine or the calcium ionophore A23187, while endothelial-independent function was not altered. These data are similar to those reported by Tsutsui et al$^{32}$ in which canine middle cerebral arteries exhibited diminished relaxation to A23187 after 24-hour incubation with 100 µmol/L sepiapterin. In that study, the authors concluded that the inhibition of endothelial-dependent relaxation was due to enhanced generation of superoxide anion, as exogenous superoxide dismutase (SOD) ameliorated the effects of sepiapterin. It is well-recognized that BH$_4$ itself can redox cycle and thus generate superoxide.$^{33}$ This effect is likely to be enhanced in the setting of vascular relaxation studies, in which the O$_2^-$ concentration is typically 95%. However, in the present report, only the hyperlipidemic vessels demonstrated a sepiapterin-dependent inhibition of endothelial-dependent relaxation. It should be noted that the normal vessels did not demonstrate an increase in BH$_4$ content after incubation with sepiapterin, while the rise in BH$_4$ content with sepiapterin supplementation did not exceed levels seen in control vessels. If the effects on vessel relaxation are secondary to BH$_4$ autooxidation, this would suggest that there is also at least a relative deficiency of SOD in the hyperlipidemic vessels. Alternatively, in the face of a functionally abnormal eNOS, could sepiapterin supplementation result in sepiapterin-dependent superoxide generation from eNOS itself, as has been described for the purified enzyme?$^{1,26}$ The effect of either exogenous SOD or a cell-permeant SOD mimetic would be informative.$^{33}$ On the other hand, BH$_4$ can serve to scavenge superoxide with a rate constant of 10$^{5}$ M$^{-1}$ · s$^{-1}$ and reacts with peroxynitrite.$^{12,19,34}$ Thus, the effects of sepiapterin are the result of extent of salvage pathway activity and conversion to BH$_4$, balanced with rates of BH$_4$ autooxidation and consumption by peroxynitrite, as well as sepiapterin binding to eNOS in competition with BH$_4$.

The inhibitory effect of 6 hours of sepiapterin treatment also contrasts with 2 studies of 8-week in vivo administration of BH$_4$ demonstrating improved endothelial function in models of SHR and diabetes.$^{28,31}$ Are these differences due to unrecognized secondary reactions of sepiapterin as opposed to BH$_4$ itself? The report by Vasquez-Vivar et al$^{39}$ opens new avenues of investigation with respect to the role of biopterins in vascular dysfunction.

References


Sepiapterin Treatment in Atherosclerosis
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